JCM
Journals.ASM.org

Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of *Candida* Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories

Teresa Spanu,[a] Brunella Posteraro,[a] Barbara Fiori,[a] Tiziana D'Inzeo,[a] Serena Campoli,[a] Alberto Ruggeri,[a] Mario Tumbarello,[b] Giulia Canu,[a] Enrico Maria Trecarichi,[b] Gabriella Parisi,[c] Mirella Tronci,[c] Maurizio Sanguinetti,[a] and Giovanni Fadda[a]

Istituto di Microbiologia,[a] Istituto di Clinica delle Malattie Infettive,[b] Università Cattolica del Sacro Cuore, Rome, Italy, and Laboratorio di Microbiologia, Azienda ospedaliera San Camillo-Forlanini, Rome, Italy[c]

We evaluated the reliability of the Bruker Daltonik's MALDI Biotyper system in species-level identification of yeasts directly from blood culture bottles. Identification results were concordant with those of the conventional culture-based method for 95.9% of *Candida albicans* (187/195) and 86.5% of non-*albicans Candida* species (128/148). Results were available in 30 min (median), suggesting that this approach is a reliable, time-saving tool for routine identification of *Candida* species causing bloodstream infection.

Bloodstream infections (BSIs) caused by yeasts can cause devastating complications and markedly increase the costs of hospital care (1). Mortality remains high, especially when effective antifungal drug therapy is not promptly administered (3, 9, 24). The prevalence of the different *Candida* species varies widely from region to region, and antifungal susceptibility patterns are species specific (18–20). Consequently, rapid, accurate identification (ID) of the causative organism is critical for successful treatment.

Blood culture (BC) remains the gold-standard method for diagnosing fungal BSI (16). On arrival in the laboratory, BC bottles are immediately loaded into automated systems that continually monitor microbial growth. When growth is detected, the BC medium is usually subcultured on agar plates to obtain isolated colonies that are used for phenotypic testing aimed at species-level ID (16), which typically takes at least 72 h (6). In order to furnish earlier results, several molecular-based methods have been evaluated for ID of yeast pathogens directly from BC bottles, but they are often time-consuming and/or require considerable expertise (10–12).

Many of the shortcomings of the older and newer methods cited above can reportedly be overcome with matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) fingerprinting based on the detection of specific proteins released from microbial cells. Compared with the conventional methods used to identify yeast isolates in clinical laboratories, MALDI-TOF MS seems to offer considerably shorter turnaround times, and thanks to the existence of a growing database of protein spectra for 110 different yeast species it may also prove to be more accurate (2, 4, 12, 15, 23).

Recently, MALDI-TOF MS has been presented as a novel method for the direct ID of yeasts from BC broth (7, 8, 14, 26). Unfortunately, all 4 studies have been relatively small, and 2 studies (8, 14) focused almost exclusively on the assessment of BC bottles inoculated with quality control strains of *Candida* spp., which may differ markedly from specimens encountered in clinical settings (5).

This study was designed to test the performance of the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonik GmbH, Leipzig, Germany) in the routine ID of yeasts directly from BC bottles submitted to two large, hospital-based microbiology laboratories in Rome, Italy.

Fungus-positive BC Bactec Mycosis IC/F bottles (Becton Dickinson Microbiology Systems, BD) obtained from November 2009 through March 2011 in the clinical microbiology laboratories of the Catholic University of the Sacred Heart Medical Center (1,300 beds) and the San Camillo-Forlanini Hospital (800 beds) were analyzed. Broth aliquots from each positive bottle were collected for pathogen ID studies, i.e., Gram staining (the results of which were immediately reported to the patient's physician); direct assay with the Bruker Biotyper and conventional testing. For control purposes, the same analyses were done on an equal number of randomly selected fungal BCs that were Bactec FX instrument (BD) negative and yielded no bacterial or fungal growth. BC broth aliquots were subcultured according to standard procedures (16), and isolates were identified to the species level by standard laboratory procedures, including morphological ID and the Vitek yeast biochemical card (YBC; bioMérieux, Marcy l'Etoile, France), as previously described (22). For Bruker Biotyper testing, clinical samples were prepared with the protocol described below, which had been defined and validated in preliminary testing of simulated BCs (Mycosis IC/F bottles inoculated with 10 ml of fresh, unprocessed blood from healthy volunteers and 10^2 cells of *Candida albicans* ATCC 18804, *Candida glabrata* ATCC 2001, *Candida guilliermondii* ATCC 6260, *Candida krusei* ATCC 6258, *Candida lusitaniae* ATCC 34449, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750). Each test was con-

Received 10 September 2011 Returned for modification 6 October 2011
Accepted 7 November 2011
Published ahead of print 16 November 2011
Address correspondence to Teresa Spanu, tspanu@rm.unicatt.it.
T. Spanu, B. Posteraro, and B. Fiori contributed equally to this article.

doi:10.1128/JCM.05742-11

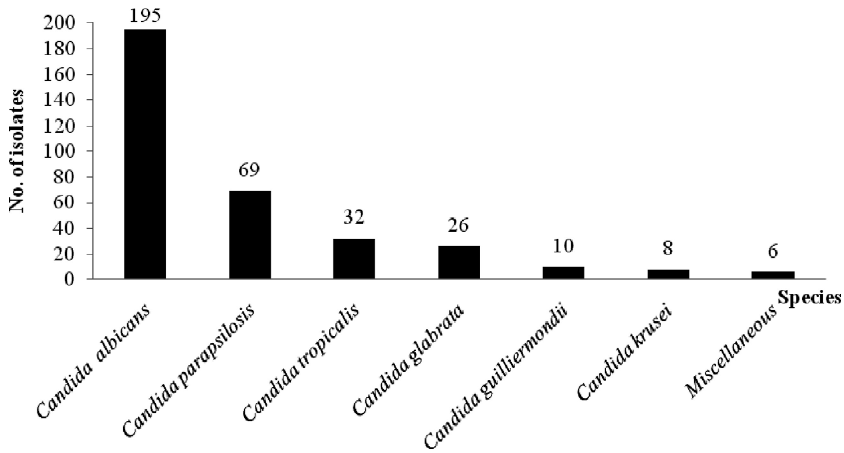

FIG 1 Distribution of isolated yeast species. Miscellaneous species include *Candida lusitaniae* ($n = 2$), *Rhodotorula mucilaginosa* ($n = 2$), *Candida famata* ($n = 1$), and *Rhodotorula glutinis* ($n = 1$). Six blood culture samples yielded two different *Candida* species, including *Candida albicans*/*Candida glabrata* ($n = 2$), *C. albicans*/*Candida krusei* ($n = 1$), *C. albicans*/*Candida parapsilosis* ($n = 1$), *C. glabrata*/*Candida guilliermondii* ($n = 1$), and *C. parapsilosis*/*Candida tropicalis* ($n = 1$).

ducted in duplicate. In accordance with the validated protocol, an 8-ml aliquot of BC broth (growth-positive or growth-negative) was centrifuged (10,000 rpm, 2 min, room temperature [RT]) and the supernatant was discarded. The pellet was washed twice with pure water (1 ml), recentrifuged, suspended in 0.1% Tween 80 (1 ml), and incubated for 2 min. After a third centrifugation, the pellet was washed twice in pure water (1 ml), recentrifuged, and suspended in pure water (300 μl) plus absolute ethanol (900 μl). This suspension was centrifuged, and 30 μl of 70% formic acid plus 30 μl pure acetonitrile were added to the pellet; the solution was thoroughly vortexed and centrifuged (14,000 rpm, 2 min, RT). The supernatant was collected for immediate testing, and aliquots were also stored at -20°C until the end of the study. Each supernatant sample (1 μl) was applied in quadruplicate to a steel MALDI target plate. The spot was dried and then overlaid with 1 μl MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid). The matrix sample was then air dried at RT for 5 min. Each testing session included a negative extraction control and the 7 ATCC strains listed above. Mass spectrometry was performed with the Microflex system (Bruker Daltonik). Captured spectra were analyzed with Bruker Biotyper software (version 2.0) and compared with those in the Biotyper database. Matches were ranked by ID log(scores) ranging from 0 to 3, and the match with the highest score was used for species ID. IDs were considered valid at the species level when $\geq$2 of the 4 spectra had log(scores) of $\geq$1.9 or when all 4 spectra had log(scores) of $\geq$1.2 (13). Bruker Biotyper and comparison method ID results were compared, and when discrepancies emerged, a definitive ID was obtained with sequence analysis of the rRNA gene internal transcribed spacer (ITS) regions, as previously described (22, 25).

In preliminary testing, the system correctly identified all ATCC *Candida* isolates with log(scores) ranging from 1.8 to 2.0. During the study period, 340 positive BCs (one sample per patient) were collected. A total of 346 yeast isolates were identified with the conventional method (Fig. 1). Direct assay of BC samples with the Bruker Biotyper identified *Candida* spp. (median time, 30 min) with high sensitivity ranging from 95.9% for *C. albicans* (95% confidence interval [CI], 91.8 to 98.1) to 86.5% for non-*albicans* *Candida* species (95% CI, 79.7 to 91.4) (Table 1). For the 316 isolates that were correctly identified, the median ID log(score) for the best hit was 1.86, and 280 (88.6%) of these isolates were identified with log(score) values of $\geq$1.81. The remaining 11.4% (36/316) had log(score) values between 1.64 and 1.79. Lower ID log(score) values were observed mainly with *C. guillermondii*, *C. krusei*, and *C. lusitaniae* isolates. For 25 samples, the Bruker Biotyper software furnished no reliable ID, with a score $<$1.2. The 30 isolates in this subgroup included 19 isolates associated with monofungal BSIs and 11 of the 12 from the 6 polyfungal samples. For all 30, ITS region sequencing confirmed the identities indicated by the comparison method: *C. albicans* ($n = 8$), *Candida famata* ($n = 1$), *C. glabrata* ($n = 4$), *C. guilliermondii* ($n = 6$), *C. krusei* ($n = 2$), *C. lusitaniae* ($n = 1$), *C. parapsilosis* ($n = 4$), *C. tropicalis* ($n = 4$), *Rhodotorula glutinis* ($n = 1$), and *Rhodotorula mucilaginosa* ($n = 2$). All 340 negative BC samples yielded negative results (specificity, 100%; 95% CI, 98.5 to 100).

TABLE 1 Performances of Bruker Biotyper for direct identification of yeasts from blood culture bottles with culture-based identification as reference

	No. of isolates		
Comparison method ID	Total tested	Concordant ID[a]	% Sensitivity (95% CI)[b]
Candida albicans	195	187	95.9 (91.8–98.1)
Candida famata	1	0	NT
Candida glabrata	26	22	84.6 (64.3–94.9)
Candida guilliermondii	10	6	60.0 (27.4–86.3)
Candida krusei	8	6	75.0 (35.6–95.5)
Candida lusitaniae	2	1	NT
Candida parapsilosis	69	65	94.2 (85.1–98.1)
Candida tropicalis	32	28	87.5 (70.1–95.9)
Rhodotorula glutinis	1	0	NT
Rhodotorula mucilaginosa	2	0	NT
Total	346	316	91.3 (87.7–93.9)

[a] Species identification furnished by the Bruker Biotyper was concordant with that of the comparison method.
[b] NT, not tested. Sensitivity was not calculated when $<$5 isolates were found.

To our knowledge, this study is the first attempt to evaluate the Bruker Biotyper's performance in the routine ID of a large number of clinical bloodstream isolates of *Candida* species. This experience confirms the results obtained in 2 studies that focused mainly on simulated fungal BCs (8, 14). In a third study (7), direct MALDI-TOF MS analysis of positive clinical BCs accurately identified bacterial pathogens but failed to provide species-level IDs for all 18 of the *Candida*-positive specimens tested. In contrast Yan and colleagues (26) recently reported that the Bruker Biotyper system correctly identified at species level all 42 BCs they tested. These studies differed methodologically from one another and from our own in a number of respects, some of which could potentially explain the discordant results obtained. The study by Ferreira et al. (7), for example, is the only one in which BC broth samples analyzed with the Bruker Biotyper were not previously subjected to detergent-induced lysis of the erythrocytes contained in the broth obtained in other studies with sodium dodecyl sulfate (14), saponin (8), MALDI Sepsityper lysis solution (26) or Tween 80 (our own). Complete removal of blood cells is essential since the proteins they contain produce spectral peaks that can mask those related to the yeasts (21). Indeed, in the preliminary phase of our study, the Bruker Biotyper displayed lower accuracy in identifying *Candida* spp. in samples that had not been treated with Tween 80 (data not shown).

The method's main weakness is its poor performance in the presence of polyfungal BSIs (fortunately, a relatively rare occurrence). For 11 of the 12 isolates associated with the 6 mixed BSIs, the Bruker Biotyper provided no ID. This is obviously preferable to providing an ID that is incorrect, but similar problems have been reported with direct MALDI-TOF MS analysis of BC broth containing multiple bacterial isolates (5, 13). This aspect clearly needs to be explored further in a larger series of multiple-isolate BCs. The *Candida* species most frequently missed by the Bruker Biotyper was *C. guilliermondii*. Other investigators have reported difficulties in identifying this species with the Bruker Biotyper, even when they used cultures grown on solid media (4, 23). It is unclear whether the problem stems from inherent characteristics of this species that reduce spectrum quality or from database entries that do not allow robust spectral matches. Inoculum size is undoubtedly a major determinant of the system's ability to identify organisms (21, 26). For fungal isolates, the best results are obtained with an inoculum of approximately 0.5×10^6 cells (21). Low fungal loads ($<10^4$ CFU/ml) were often observed in monofungal samples for which the system did not furnish reliable IDs, suggesting a suboptimal analyte concentration as the most relevant cause of unsuccessful direct ID.

Although all the IDs proposed by the Biotyper were correct, their log(score) values were consistently lower than those reported when yeast isolates are subcultured on solid media (2, 4, 15, 23). The scores for close to 90% of our samples were ≥ 1.8, the threshold for species-level ID proposed for routine clinical testing of subcultured yeast isolates (4, 23), and in the remaining 10%, log(score) values ranged from 1.64 to 1.79. Log(score) values have not been reported in 2 previous reports on direct ID of yeasts in BC broths (8, 14), while Yan and colleagues (26) identified at the species level all BCs with log(score) values >1.9.

One of the shortcomings of our study is the limited diversity of yeast genera and species we examined. However, this limitation is to some extent outweighed by the fact that our cohort was quite large and it included the yeast pathogens most commonly responsible for BSIs (18–20). Nonetheless, further investigation is needed to evaluate the Biotyper's reliability for direct ID of yeast pathogens in BC broth. Second, our study was not designed to address costs. Although use of the Bruker Biotyper is undeniably associated with added expenses, the cost of the instrument and its maintenance are offset by the low costs of the consumables (4). The costs will also need to be weighed against other potential savings related to the earlier ID of candidemia (e.g., shorter hospital stays, targeted investigation, more favorable case outcomes), although these will also have to be defined in future studies.

In conclusion, our study confirms that the Bruker Biotyper system is one of the more promising alternatives proposed to accelerate species-level ID of yeast isolates. Furthermore, almost 80% of our positive BCs were reported positive $\leq$24 h after sample entry (median time, 16 h). Consequently, with direct Bruker Biotyper assay of BC broth, physicians can realistically expect (in many cases) to receive species-level ID data for *Candida* isolates causing BSI within 24 h after the BC is drawn, a prospect that is unthinkable with conventional culture-based phenotypic testing. This advantage, together with other time-saving measures (e.g., prompt collection of BCs when BSI is first suspected, immediate transport of cultures to the laboratory), should allow considerably earlier prescription of effective drug therapy for yeast BSIs, with positive effects on patient outcomes (9, 17, 24).

ACKNOWLEDGMENTS

We thank Marian Everett Kent for her assistance in editing the manuscript.

This work was supported by a grant from the Università Cattolica del Sacro Cuore (Fondi Ateneo, Linea D1-2009). We have no conflicts of interest to declare.

REFERENCES

1. **Arnold HM, et al.** 2010. Hospital resource utilization and costs of inappropriate treatment of candidemia. Pharmacotherapy. **30**:361–368.
2. **Bader O, et al.** 2011. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Microbiol. Infect. **17**:1359–1365.
3. **Bassetti M, et al.** 2007. Incidence, risk factors, and predictors of outcome of candidemia. Survey in 2 Italian university hospitals. Diagn. Microbiol. Infect. Dis. **58**:325–331.
4. **Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL.** 2011. Performance and cost analysis of matrix-assisted laser desorption ionization–time of flight mass spectrometry for routine identification of yeast. J. Clin. Microbiol. **49**:1614–1616.
5. **Drancourt M.** 2010. Detection of microorganisms in blood specimens using matrix-assisted laser desorption ionization time-of-flight mass spectrometry: a review. Clin. Microbiol. Infect. **16**:1620–1625.
6. **Fernandez J, Erstad BL, Petty W, Nix DE.** 2009. Time to positive culture and identification for *Candida* blood stream infections. Diagn. Microbiol. Infect. Dis. **64**:402–407.
7. **Ferreira L, et al.** 2011. Microorganisms direct identification from blood culture by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Clin. Microbiol. Infect. **17**:546–551.
8. **Ferroni A, et al.** 2010. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **48**:1542–1548.
9. **Garey KW, et al.** 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. **43**:25–31.
10. **Gherna M, Merz WG.** 2009. Identification of *Candida albicans* and *Candida glabrata* within 1.5 hours directly from positive blood culture bottles with a shortened peptide nucleic acid fluorescence in situ hybridization protocol. J. Clin. Microbiol. **47**:247–248.
11. **Hsiue HC, et al.** 2010. Rapid identification of fungal pathogens in positive

blood cultures using oligonucleotide array hybridization. Clin. Microbiol. Infect. **16**:493–500.

12. **Kaleta EJ, et al.** 2011. Comparative analysis of PCR-electrospray ionization/mass spectrometry (MS) and MALDI-TOF/MS for the identification of bacteria and yeast from positive blood culture bottles. Clin. Chem. **57**:1057–1067.
13. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4**:e8041.
14. **Marinach-Patrice C, et al.** 2010. Rapid species diagnosis for invasive candidiasis using mass spectrometry. PLoS One **5**:e8862.
15. **Marklein G, et al.** 2009. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47**:2912–2917.
16. **Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (ed).** 1999. Manual of clinical microbiology, 7th ed. ASM Press, Washington, DC.
17. **Pappas PG, et al.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **48**:503–535.
18. **Pfaller MA, Diekema DJ.** 2002. Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. J. Clin. Microbiol. **40**:3551–3557.
19. **Pfaller MA, Diekema DJ.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20**:133–163.
20. **Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M.** 2011. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). J. Clin. Microbiol. **49**:396–399.
21. **Qian J, Cutler JE, Cole RB, Cai Y.** 2008. MALDI-TOF mass signatures for differentiation of yeast species, strain grouping and monitoring of morphogenesis markers. Anal. Bioanal. Chem. **392**:439–449.
22. **Sanguinetti M, et al.** 2007. Evaluation of VITEK 2 and RapID yeast plus systems for yeast species identification: experience at a large clinical microbiology laboratory. J. Clin. Microbiol. **45**:1343–1346.
23. **Stevenson LG, Drake SK, Shea YR, Zelazny AM, Murray PR.** 2010. Evaluation of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of clinically important yeast species. J. Clin. Microbiol. **48**:3482–3486.
24. **Tumbarello M, et al.** 2007. Biofilm production by *Candida* species and inadequate antifungal therapy as predictors of mortality for patients with candidemia. J. Clin. Microbiol. **45**:1843–1850.
25. **White TJ, Bruns T, Lee S, Taylor J.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315–322. *In*Innis MA, Gelfand DH, Sninsky JJ, White TJ (ed), PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA.
26. **Yan Y, et al.** 2011. Improved identification of yeast species directly from positive blood culture media by combining Sepsityper specimen processing and Microflex analysis with the matrix-assisted laser desorption ionization Biotyper system. J. Clin. Microbiol. **49**:2528–2532.